

Efficacy of Washing and Sanitizing Trailers Used for Swine Transport in Reduction of *Salmonella* and *Escherichia coli*

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ABSTRACT

Healthy pigs can carry *Salmonella* in their intestine and may shed this pathogen because of stresses incurred during transportation, contaminating trailer floors and bedding material. If not cleaned and sanitized between trips, trailers and bedding have the potential to infect other farms, the abattoir environment, or other animals with *Salmonella*. Floors and bedding material from pig trailers were sampled to determine the efficacy of the abattoir-developed washing and sanitizing regime on the level of *Salmonella* before and after a single haul. *Escherichia coli* levels were an indicator of high contamination. The study also determined the effect of ambient temperature (during four seasons) and of the distance the pigs traveled in the haulers (>500 miles or <500 miles) on bacterial levels. *Salmonella* was isolated from 80% of the bedding material tested. Of the 188 floor samples taken, 41.5% were positive for *Salmonella* before washing, and 2.7% were positive after washing and sanitizing. *E. coli* was isolated from all bedding material and floor samples before washing, but washing and sanitizing significantly decreased levels ($P < 0.05$) by 2 logs. There was no significant difference ($P > 0.05$) in the number of *Salmonella*- or *E. coli*-positive trailers attributable to distance traveled or season of the year. These results demonstrate that washing and sanitizing the trailers after each load significantly reduced levels of *Salmonella* and its possible spread by the contaminated trailer and bedding, which ultimately could promote improvement in food safety.

During handling and transportation poultry, livestock, and other animals become stressed (9, 21, 24) because of changes in their environment, group size, loading and unloading practices, and distance traveled (13). These stresses can result in shipping fever for cattle and horses (16, 17, 24) and even death during or shortly after transportation for pigs (13). There is increased excretion of fecal material by all species studied (9, 13).

Salmonella was first isolated from pigs in 1889 (26). Although some healthy pigs are reported to carry *Salmonella* in their intestinal tracts without shedding (12), shedding of this pathogen may occur under the stress of being transported (27). *Salmonella*-contaminated trucks may infect other farms (7), abattoirs (11), and other animals (5, 6) if the trailers are not cleaned and the bedding material is not removed and replaced between trips.

Handling practices between farm and slaughter have been shown to influence the recovery of *Salmonella* from carcasses (14, 15, 20). When a pig becomes contaminated with *Salmonella* during transportation or slaughter, the carcass and resulting meat can become a source for salmonellosis in humans (10, 18, 23, 27). *Salmonella* has been isolated from raw pork in butcher shops (8, 18), from raw boneless Canadian back pork loins (1), and from fresh and

smoked pork sausage involved in a foodborne outbreak of salmonellosis (10).

Previous research has indicated that washing and decontamination can reduce bacteria counts on poultry cages (4) and in aircraft and ships used for livestock transport (2). In a study by Childers et al. (3), were cleaned and sanitized before the pigs were transported, but no reduction in bacterial level in the trailers was reported. There are no published reports of the microbiological effect of washing and sanitizing of live swine trailers. The primary objective of this study was to determine the efficacy of washing and sanitizing pig trailers between loads to reduce or eliminate *Salmonella* incidence and levels. A secondary objective was to determine if season of the year and distance traveled affected the levels of *Salmonella* and *Escherichia coli* found in the trailers before washing.

MATERIALS AND METHODS

Trailer description. The trailers were part of the Hatfield, Inc. fleet (Hatfield, PA) and were identical in layout and floor area. The trailer floors were constructed of aluminum. Each trailer was constructed in three tiers that were divided, by gates, into 10 pens. Each pen can hold up to 20 pigs, with a maximum of 200 pigs in each trailer. The pens were labeled from front to back as follows: A and B (bottom tier); C, D, E, and F (middle tier); and G, H, I, and J (top tier). All pigs were loaded and unloaded through pen F. An estimate of variance on preliminary data determined that six pens (A, B, D, E, F, and J) needed to be sampled for the study.

Before leaving the plant, the floor in each washed and sanitized trailer was covered with fresh sawdust bedding. Each trailer carried a single load (one trip) before sampling. The hauling

distances were grouped either as short haul (<500 miles) or long haul (>500 miles) to indicate the length of time the pigs spent in the trailer. The study was conducted over 17 months in southeast Pennsylvania, and the daily (6:00 A.M.) outdoor temperature was recorded at the plant. A total of 32 trailers were sampled at the following times: spring (average 10.8°C), 2 short and 3 long hauls; summer (average 19.4°C), 2 short and 8 long hauls; fall (average 10.7°C), 2 short and 5 long hauls; and winter (average 0.8°C), 2 short and 8 long hauls.

Sampling trailer floors. Pigs from commercial growers were loaded by the Hatfield driver, after initial company training on handling the animals in the least stressful manner, into the clean trailers for direct transport to the slaughter plant. Upon arrival at the plant, the driver immediately unloaded the pigs. The trailer was then moved to Hatfield's trailer-washing facility for sampling. Bedding materials from the pens' sampling areas were aseptically collected (regloving after each sampling step) and placed in a Whirl-pack bag (Nasco, Fort Atkinson, WI) for the trailer's composite bedding sample. The before-washing trailer samples were taken. The floor areas to be sampled were aseptically cleared of the bedding material by brushing it aside with a sterile gloved hand. A sterile sponge from a Whirl-pack bag (10 × 20 cm bag containing a 3.7 × 7.4 cm sponge), dampened with 10 ml of 1% buffered peptone water (BPW; Difco Laboratories, Detroit, MI), was used to swab the floor area once using a sterile 100-cm² sterile template as guide. The sponge was returned to the bag, labeled before washing, and stored refrigerated until it was analyzed (within 3 h). The trailer was washed immediately. After the washing and sanitizing were completed, but before rebedding, the above floor sampling procedure was repeated to obtain after-washing floor swabs from the same five to six pen area previously sampled.

Trailer washing. The trailer was manually cleaned and sanitized by the Hatfield personnel in their trailer-washing facility, which was constructed to accommodate four trailers. The floor is graded to improve drainage of liquids from the trailers. The liquids used, except where stated, were at ambient temperature. The force and volume of the liquids from the hoses were the only cleaning action used. The procedure established by Hatfield was as follows: (a) rinse with chlorinated (chlorine level of 3 ppm) reconditioned water (19) to physically remove dirt and bedding material; (b) rinse with potable water; (c) wash with alkaline detergent diluted 1:10 (Power Play; Equipment Trade Service Co., Norwood, PA); (d) rinse with heated (80 ± 10°C) potable water; (e) spray interior with quaternary ammonium sanitizer (1:400 dilution of Roccal-D; The Upjohn Co., Kalamazoo, MI); and (f) cover trailer floor with five to six bags (50–70 lb) of fresh sawdust bedding (Tyson Foods, New Holland, PA).

Microbiological analysis. After the addition of 90 ml of 1% BPW, the sponge samples were mixed for 1 min at normal speed using a Stomacher (Stomacher 400; Tekman, Cincinnati, OH). For the composite bedding, a 10.0-g sample was weighed, 90 ml of BPW was added, and the sample was mixed for 1 min at normal speed.

***E. coli* biotype 1.** After the mixed composite bedding sample and the sponge sample were serially diluted using 0.1% peptone water (Difco), *E. coli* estimates were obtained using Petrifilm *E. coli* count plates (3M Microbiology Products, St. Paul, MN) and following the manufacturer's recommended procedures. The Petrifilms were hand counted after incubation at 37°C for 24 h and again after 48 h of incubation.

Salmonella. To quantitate the levels of *Salmonella*, a three-tube most probable number (MPN) technique was used. The mixed sponge and bedding samples were serially diluted using BPW. The assay procedure included (a) preenrichment in 1% BPW incubated for 24 h at 37°C; (b) selective enrichment in tetrathionate broth (Difco) incubated for 18–24 h at 42°C and selenite cystine broth (Difco) incubated for 18–24 h at 37°C; (c) presumptive identification of characteristic colonies after streaking of the selective enrichment broth on BG sulfa and double-modified lysine iron agars (Difco); (d) purification and isolation by selection of typical positive colonies and restreaking onto XLD or XLT-4 agars (Difco); and (e) confirmation using API 20E biochemical test strips (bioMérieux Vitek, Inc., Hazelwood, MO) and antigenic profile using *Salmonella* O antiserum poly A and B and *Salmonella* H antiserum poly a–z agglutination (Difco).

After the selective enrichment incubation step of the MPN procedure was completed, the samples were screened using the TECRA *Salmonella* visual immunoassay (Inter. BioProducts, Inc., Redmond, WA). If results were positive, the MPN procedure was continued with streaking of the broths onto the agars.

Statistical analysis. An estimate of variance components was performed to determine the pen number and number of samples per trailer needed for this study. χ^2 Analysis was used to investigate the effect of season on the rate of incidence of positive *Salmonella* and *E. coli* results in the bedding samples. ANOVA was used to determine the significance of the effects and interactions of the season of the year and washing and sanitizing of the trailers between loads on *Salmonella* and *E. coli* levels. The effect of washing and sanitizing the trailers on the rate of incidence of positive *Salmonella* and *E. coli* results was investigated by using χ^2 analysis and the sign test. χ^2 Analysis and Fisher's exact test were performed to determine any significant effect of distance traveled (22).

RESULTS

Bedding material samples. Composite bedding samples from 30 of the 32 trailers were collected, and the *Salmonella* and *E. coli* levels were determined. All samples were positive for *E. coli* (Table 1), and the levels found in the bedding ranged from <1 to 8.4 log CFU/g. The mean ± SD of *E. coli* levels were 5.3 ± 0.9, 6.9 ± 0.6, 6.3 ± 0.4, and 4.8 ± 1.5 log CFU/g for spring, summer, fall, and winter, respectively. Season of year and distance traveled had no significant effect ($P > 0.05$) on the *E. coli* level recovered from the bedding samples.

Salmonella was isolated and confirmed in 24 of 30 (80%) bedding samples tested; levels ranged from 1 to >110 MPN/g. The recovery incidence of *Salmonella* was reduced to 50% (5 of 10) during the winter months compared with 100% for spring, 88% for summer, and 100% for the fall months (Table 1). This reduction in number of bedding samples during the winter was statistically significant ($P < 0.05$, as determined by χ^2 analysis). When the *Salmonella* level recovered from the bedding samples were compared statistically, there was no significant effect ($P > 0.05$).

Trailer floor samples. A trailer was considered positive for *Salmonella* if the isolates from any of the six pens were confirmed. Of the 32 trailers tested before washing, *Salmonella* was isolated from 25 trailers (78%), with at least one pen positive (data not shown). The *Salmonella* level

TABLE 1. Effect of season of the year on levels of *E. coli* and *Salmonella* in composite bedding samples from trailers

| Season | No. of bedding samples tested | <i>E. coli</i> ^a | | | <i>Salmonella</i> ^b | | |
|--------|-------------------------------|-----------------------------|---------|-------------------|--------------------------------|--------|-------------------|
| | | No. positive | Range | Mean ^c | No. positive | Range | Mean ^c |
| Spring | 5 | 5 | 2.9–8.4 | 5.3 ± 0.9 | 5 | 1–110 | 22.8 ± 43.6 |
| Summer | 8 | 8 | 5.4–8.1 | 6.9 ± 0.6 | 7 | 1–110 | 26.1 ± 37.6 |
| Fall | 7 | 7 | 5.1–6.9 | 6.3 ± 0.4 | 7 | 1–>110 | 54.4 ± 50.3 |
| Winter | 10 | 10 | <1–6.3 | 4.8 ± 1.5 | 5 | 1–110 | 34.8 ± 41.0 |

^a log cfu/g.^b MPN/g; detectability, 1 MPN/g.^c Overall no significant effect of season or distance traveled, $P > 0.05$.

recovered in some trailers had >110 MPN/cm² of floor area (Table 2). There were a total of 188 before-washing pen samples, of which 41.5% (78 pens) yielded confirmed *Salmonella* isolates. Overall, season of the year (Table 2) and distance traveled (Table 3) had no significant effect ($P > 0.05$) on the before-washing *Salmonella* level recovered. When the sign test was used on instances of nonzero count before washing, there was evidence of significant ($P < 0.05$) reduction in counts by washing. After washing and sanitizing (Table 2), 5 of 188 pens sampled (2.7%) contained *Salmonella*. In all other cases, *Salmonella* was reduced to undetectable levels (<1 MPN/cm²) after washing and sanitizing.

All trailers were positive for *E. coli* before washing and sanitizing, and some trailer floors had levels as high as log 5 CFU/cm². Overall, season of the year and distance traveled

had no statistical influence ($P > 0.05$) on the incidence of *E. coli* recovered (Tables 2 and 3). After washing and sanitizing, except for three pens in the spring sampling, overall there was an average 2 log reduction in *E. coli* counts (Tables 2 and 3). The recovery from these three pens was higher than before washing and was attributed to recontamination by the sampler before the sample was taken. Of the after-washing samples, 34 of 181 pens sampled (18.8%) were positive for *E. coli*. The reduction attributable to washing and sanitizing was statistically significant ($P < 0.05$); a mean prewash level of 3.4 cfu/cm² was lowered to a mean postwash level of 1.4 cfu/cm².

DISCUSSION

Cancellotti (2) reviewed washing procedures and disinfectants used on airliners and ships that transport animals.

TABLE 2. Effect of season on recovery levels of *Salmonella* and *E. coli* from the floors of trailers

| Season | Trailers tested (n) | Pens tested (n) | <i>Salmonella</i> ^a | | | | | | <i>E. coli</i> ^b | | | | | |
|--------|---------------------|-----------------|--------------------------------|--------|-------------|----------------------------|--------|-------------|-----------------------------|--------|-----------|----------------------------|--------|-----------|
| | | | Before washing | | | After washing ^c | | | Before washing | | | After washing ^c | | |
| | | | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean |
| Spring | 5 | 30 | 8 | 1–21 | 8.3 ± 6.6 | 2 | 1–21 | 11.0 ± 10.0 | 28 | <1–4.4 | 2.4 ± 1.0 | 5 | <1–3.4 | 2.5 ± 0.8 |
| Summer | 10 | 56 | 27 | 1–>110 | 11.2 ± 28.1 | 1 | 2 | 2 ± 0 | 56 | <1–5.7 | 4.2 ± 1.1 | 10 | <1–3.7 | 1.4 ± 1.0 |
| Fall | 7 | 42 | 26 | 1–>110 | 37.2 ± 48.9 | 2 | 1–>110 | 55.5 ± 54.5 | 42 | <1–5.3 | 3.8 ± 1.1 | 10 | <1–1.5 | 1.3 ± 0.3 |
| Winter | 10 | 60 | 17 | 1–24 | 2.9 ± 5.6 | — ^d | — | — | 55 | <1–4.6 | 2.6 ± 1.0 | 9 | <1–1.3 | 1.0 ± 0.4 |
| Total | 32 | 188 | 78 | | | 5 | | | 181 | | | 34 | | |

^a MPN/cm²; detectability, 1 MPN/cm².^b Log cfu/cm².^c After-washing levels were significantly lower ($P < 0.5$) than before-washing levels.^d Negative for *Salmonella* (<1 MPN/cm²).TABLE 3. Effect of distance traveled on recovery levels of *Salmonella* and *E. coli* from the floors of trailers

| Distance | Trailers (n) | Pens (n) | <i>Salmonella</i> ^a | | | | | | <i>E. coli</i> ^b | | | | | |
|----------|--------------|----------|--------------------------------|--------|-------------|----------------------------|-------|-------------|-----------------------------|---------|-----------|----------------------------|---------|-----------|
| | | | Before washing | | | After washing ^c | | | Before washing | | | After washing ^c | | |
| | | | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean | Positive pens (n) | Range | Mean |
| Short | 8 | 48 | 15 | 1–110 | 11.0 ± 27.1 | 2 | 1 | 0 ± 0 | 48 | <1–5.65 | 3.7 ± 1.2 | 11 | <1–3.90 | 1.5 ± 0.8 |
| Long | 24 | 140 | 63 | 1–>110 | 19.4 ± 28.1 | 3 | 2–110 | 44.3 ± 47.1 | 140 | <1–5.73 | 3.2 ± 1.3 | 21 | <1–3.45 | 1.4 ± 0.8 |

^a MPN/cm²; detectability, 1 MPN/cm².^b Log cfu/cm².^c After-washing levels were significantly lower ($P < 0.5$) than before-washing levels.

He suggested that with proper washing and sanitizing, these procedures can be effective in removing more than 90% of the microorganisms present to provide a less stressful environment, thus ensuring the arrival of healthy animals (2). The washing and sanitizing procedures for the nonporous surfaces of trailers used in this study is similar or greater than published recommendations for *Salmonella* and *E. coli* reduction.

In clinically healthy pigs carrying *Salmonella*, excretion patterns for this pathogen can change from intermittent to constant shedding if an external stress, such as transportation, upsets the equilibrium of the pigs' intestinal flora (26, 28). This type of stress is responsible for an increase in the "carrier" state of *Salmonella* for pigs as seen by an increase in rectal swab isolation and after slaughter testing (21). When transportation stress occurs, shedding of the microorganism begins immediately, resulting in contaminated trailers (28). Results from this study confirmed these previous reports because trailers were positive for *Salmonella* after transportation of the pigs.

Reduction and spread of *Salmonella* in the preslaughter environment may also affect contamination rates on carcasses (21). In a study by Childers et al. (3), trailers were cleaned and sanitized. The investigators do not state how trailers were washed and sanitized, nor do they report on levels of *Salmonella* or *E. coli* obtained from the trailers. However, they do show a reduction in both carcass bacteria levels obtained from the anatomical midline before evisceration and from the body cavity for those pigs transported in a chlorophenylphenate-sanitized trailer and holding pen. Research on poultry cages also indicate the efficacy of washing and sanitizing in reducing *Salmonella* and possible spread to pathogen-free poultry farms (4). Data from this study also showed, after washing and sanitizing, reduction to undetectable levels of *Salmonella* and a >2 log reduction of *E. coli*.

Watkins and Sleath (25) reported that *Salmonella* and *E. coli* can be isolated from washed cattle lorry effluents. They also reported that when positive sewage sludge was applied to land, *Salmonella* required up to 3 weeks to reach undetectable levels. This latter finding suggests that if *Salmonella*-positive bedding drops from a trailer onto the farm or in the abattoir, *Salmonella* could survive and become a means of infecting *Salmonella*-free pigs. It could be spread to other environments just from walking across contaminated areas. *Salmonella*-free pigs can acquire this pathogen just from being exposed to such a contaminated environment, and because pigs are social animals, nose-to-nose contact only increases the rate of transmission (7). Gray et al. (12) demonstrated that only a few pigs shedding low levels of *Salmonella* can result in rapid transmission and subsequent shedding by many. The bedding samples in the present study were shown to be *Salmonella* positive. By removal after each trip, the possibility of the bedding dropping and becoming an infection vector is eliminated, further reducing the risk of contaminating a *Salmonella*-free farm or other area.

The limited reduction of *E. coli* compared with *Salmonella* observed in the present study can be explained by the differences in sensitivity of these two bacteria to the

sanitizer used. The sanitizer used in this study, according to the manufacturer's information, was not effective against all enterobacteria as seen by only a 2-log reduction in *E. coli* levels. In only one case the postwashed level of *E. coli* was higher than the prewashed, indicating the possibility of recontaminating the cleaned trailer if care is not taken to wash the boots.

There was a decrease in *Salmonella* levels recovered from the bedding and floor samples during the winter months. In the present study, the seasonal incidence of both bacteria recovered from the floor samples showed no statistical difference ($P > 0.05$). This may be explained by freezing of feces during the winter months, which would solidify onto the bedding and would not reach the cold truck floor.

In summary, in this study, we showed that the bedding material from hog-hauling trailers was positive for *Salmonella* and *E. coli* and that washing and sanitizing after animal unloading significantly reduced the incidence of *Salmonella* and *E. coli* found in the trailers. This intervention could be expected to reduce the potential for spreading and cross-contaminating other animals, farms, and the slaughter environment.

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